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Application No. 10/049,975
Filed: October 1, 2002
Confirmation No.: 6238
TC Art Unit: 1641

REMARKS

The Applicants acknowledge that the Examiner has made the restriction requirement final. The claims not drawn to the claim group being examined are hereby withdrawn. However, the Applicants reserve their right to add back the withdrawn claims and argue later for rejoinder.

Pending claims 1-6, 27-37, 45-47, 52 and 54 (claims 53 and 55 having been cancelled in Applicants' Response to Restriction Requirement dated December 21, 2005) have been rejected under 35 U.S.C. § 112 for indefiniteness and lack of enablement. These rejections are respectfully traversed in light of the claim amendments and the remarks given below. Reconsideration is requested.

Claim 1 as amended herein is supported at least at p. 4, lines 1 to 8, and p. 4, line 34 to p. 5, line 8 of the English translation of the specification. Applicants submit that amended claim 1 and the claims depending thereon are definite and are enabled by the specification. Specifically, it should be noted that the subject matter of claim 1 of the present application is disclosed in a full, clear and concise manner that is sufficient for the method to be carried out by a person skilled in the art. In particular, it is disclosed on, e.g., the bridging paragraph of pp. 8 and 9 as well as in Figs. 6a and 6b of the present application how to choose the concentration of a dye in order to assay substances according to the method characterized in present claim 1. Therefore, the teaching of the present application enables a person skilled in the art to successfully carry out the method as defined in present claim 1, particularly concerning the

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use of a suitable dye as well as the use of a suitable concentration thereof.

Concerning the Examiner's comment regarding claim 2, claim 2 has been amended to conform to the wording of this claim in the original English translation, the Applicants' submission of December 21, 2005, containing an incorrect wording of claim 2. This amendment is also supported in the specification as filed. See p. 4, line 15 of the English translation. Thus, Applicants submit that the rejection has been overcome.

Regarding claim 4, the Applicants submit that claim 4, as amended, is supported in the specification at least at p. 4, lines 16-17, of the English language translation. Thus, the rejection of claim 4 has been overcome.

Claim 27 has been amended to replace the wording "selected from the group of" by the wording "selected from the group consisting of." Applicants, therefore, submit that the rejection of claim 27 has been overcome.

Concerning the rejection of claim 28, claim 28 has been amended and new claim 56 added to address the concerns of the Examiner.

Concerning the rejection of claim 31, claim 31 has been amended and new claim 57 added to address the concerns of the Examiner. The Applicants submit that it is a well known fact to those skilled in the art that the trademarked compounds Cryptofluor Crimson and Cryptofluor Red contain as active agents phycobilins isolated from cryptomonad algae (cf. product information obtained from www.sigma-aldrich.com, a copy of which is enclosed). Therefore, the wording "Cryptofluor Crimson or Cryptofluor Red" has been replaced by the wording "low-molecular

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weight cryptomonad-derived phycobili proteins" in new dependent claim 57.

Regarding the objections raised by the Examiner against the alleged use of trademark compounds in the subject matter of present claim 32, it should be noted that it is clear to a person skilled in the art that Cy5 is not a trademark but an abbreviation for 5-N-N'-diethyltetramethylindodicarbocyanine (cf., for example, the abstract of Majumdar et al. (2001) Biophys. J. (Annual Meeting Abstracts) 80 (1): 483, a copy of which is enclosed), and claim 32 has been amended to incorporate this name.

Concerning the additional objection to claim 32, it is first pointed out that the acronym "BODIFY" should read "BODIPY." This error was introduced in the English translation. Original claim 12 of the German specification contains the correct acronym. Applicants submit that it is clear to a person skilled in the art that BODIPY is not a trademark but an acronym for dipyrromethene boron difluoride (cf., www.thefreedictionary.com, a copy of which is enclosed). Claim 32 has been so amended. Thus, Applicants submit that the rejection of claim 32 has been overcome.

Regarding claim 37, it is clear to a person skilled in the art that Brilliant Blue FCF is not a trademark but a trivial name for disodium alpha-(4-(N-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-3-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene-2-sulfonate (cf., for example, www.saujanya.com, a copy of which is enclosed). Claim 37 has been so amended.

Claim 47, an "omnibus" claim, has been amended and new claims 60-62 have been added in parallel with earlier discussed claims.

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Applicants submit that the rejection of claim 47 has been overcome.

Claims 45, 46, 52 and 54 have been rejected as indefinite and as lacking utility. The indicated claims have been amended as suggested by the Examiner, and it is submitted that the rejections have been overcome.

Applicants submit that all claims are in condition for allowance and such action is respectfully requested.

The Examiner is encouraged to telephone the undersigned attorney to discuss any matter that would expedite allowance of the present application.

Respectfully submitted,

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Appl. Serial No: 10/049,975

17861 Biotin-CryptoFluor™ Crimson

Fluka BioChemika, for fluorescence

MDL number MFCD03097680

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Price and Availability

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Descriptions

General description

The CryptoFluor™ dyes are phycobiliproteins isolated from cryptomonad algae that provide additional spectral characteristics to complement the more common phycobiliproteins (e.g., R-PE and APC) as well as providing much smaller molecular weights (around 40,000 Daltons). These characteristics are meant to provide additional functionality to the phycobiliprotein pigments when it comes to multiplexing assays or to getting dyes into the cell.

Packaging

Reconstitution

One vial contents 0.5 mg of conjugate and ~11 mg additives (see reconstitution)
Reconstitute to 250 μ l with distilled or deionized water (the solution will consist of 100 mM sodium phosphate, pH 7.2, 50 mM sucrose, 0.05% sodium azide, 150 mM sodium chloride, 0.1% bovine serum albumin and 2 mg/ml pigment. Do not freeze, store at 4 °C in the dark.

Properties

grade

for fluorescence

product line

BioChemika

mol wt

$M_r \sim 40200$ ($\alpha\alpha'\beta_2$)

fluorescence

λ_{ex} 585 nm; λ_{em} ~658 nm in 0.1 M phosphate pH 7.2

storage temp.

2-8°C

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Fluorescent Properties of Cy5 (5-N-N'-diethyl-tetramethylindodicarbocyanine) covalently linked to DNA

Zigurts K Majumdar, Maria Lucia D'Amico, Robert M Clegg: University of Illinois Urbana Champaign, Physics/LFD, Loomis Laboratory of Physics 1110 W. Green St., Urbana, IL 61801

Fluorescent dyes covalently linked at specific sites on nucleic acids are used for determining various properties of the nucleic acids, such as their structure and stability by the use of FRET. Photophysical studies lead to a better understanding of the interactions of molecules with the chromophore. The Cy3-Cy5 pair is an optimal donor-acceptor pair for FRET. Both dyes have high extinction coefficients (150,000 and 250,000 cm^3M^{-1} , respectively) and their emission spectra are well separated from most sample auto-fluorescence. We present an analysis of the fluorescence properties of Cy5 attached to the 5' end of double-stranded (ds) DNA with a C3-linker to the phosphodiester. Measurements of the fluorescence lifetime, time-resolved anisotropy and steady state measurements are used to understand the structure of the Cy5-DNA complex. Temperature and solvent effects on the fluorescence properties are studied as well. In addition to providing a better understanding of the structure of the Cy5-DNA complex, these measurements are compared to the analogous properties of Cy3 (5-N-N'-diethyl-tetramethylindodicarbocyanine) covalently attached to dsDNA with the same linker. This work was supported in part by the NIH, PHS 5 P41 RR03155.

thefreedictionary.com

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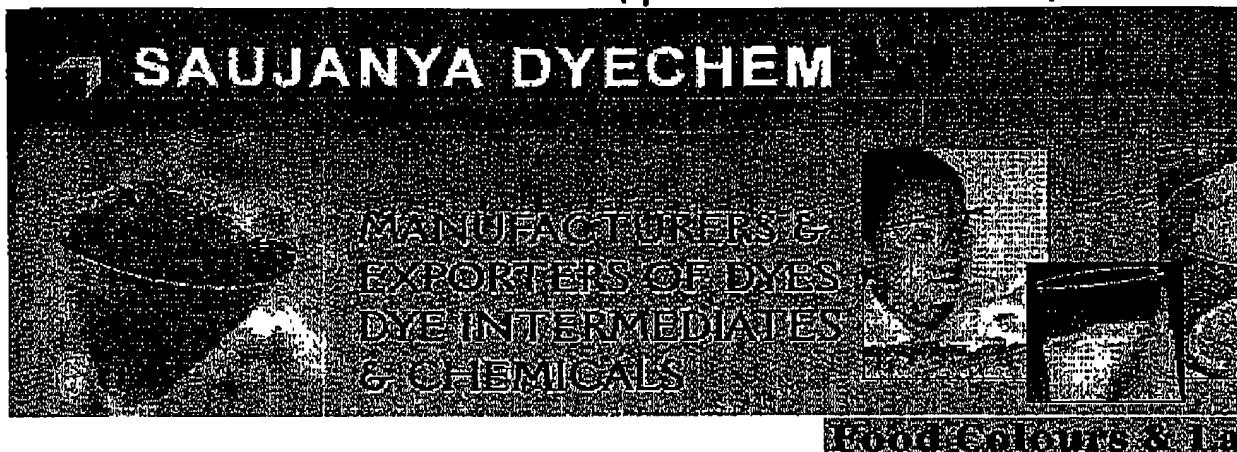
Acronym Definition

BODIPY DIPYrrromethene BOron Difluoride

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BRILLIANT BLUE FCF

SYNOMYS	BRILLIANT BLUE FCF * C. I. FOOD BLUE 2 * FD & C BLI ACID BLUE 9
DEFINITION	Brilliant Blue FCF consists essentially of disodium alpha-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene sulfonate and its isomers and subsidiary colouring matter together with sodium chloride and/or sodium sulfate as principal uncoloured components. Brilliant Blue FCF is described as the sodium salt. The sodium and the potassium salt are also permitted.
CLASS	Triarylmethane
COLOUR INDEX NO.	42090
EEC NUMBER	E-133
EINECS	223-339-8
CHEMICAL NAMES	Disodium alpha -(4-(N-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-3-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene-2-sulfonate
CHEMICAL FORMULA	$C_{37}H_{34}N_2Na_2O_9S_3$
MOLECULAR WEIGHT	792.84
ASSAY	Content not less than 85% total colouring matters, calculated on the sodium salt; E 1% 1cm 630 at ca 630 nm

DESCRIPTION	Reddish-blue powder
IDENTIFICATION	
a. Spectrometry b. Blue solution in water	Maximum in water at ca 630 nm
PURITY Water Insoluble matter	Not more than 0.2%
Subsidiary colouring matters	Not more than 6.0%
Organic compounds other than colouring matters	[Sum of 2-, 3- and 4-formyl benzene sulfonic acids] - Not more than 1.5% [3(ethyl)(4-sulfophenyl)amino) methyl benzene acid] - Not more than 0.3%
Leuco base	Not more than 5.0%
Unsulfonated primary aromatic amines	Not more than 0.01% (Calculated as aniline)
Ether extractable matter	Not more than 0.2% at pH7
Arsenic	Not more than 3 mg/kg.
Lead	Not more than 10 mg/kg.
Mercury	Not more than 1 mg/kg.
Cadmium	Not more than 1 mg/kg.
Heavy metals (as Pb)	Not more than 40 mg/kg.

||| Food Colours |||

|| TARTRAZINE || SUNSET YELLOW FCF || PONCEAU 4R || CARMOISINE || INDIGO CARMINE || ERYTHROSINE
 || AMARANTH || QUINOLINE YELLOW-WS || BRILLIANT BLUE FCF || CHOCOLATE BROWN ||
 || ALLURA RED || PATENT BLUE V || FOOD GREEN S || BLACK PN || FAST RED E ||

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BRILLIANT BLUE FCF

SYNOMYS	BRILLIANT BLUE FCF * C. I. FOOD BLUE 2 * FD & C BLUE 1 * ACID BLUE 9
DEFINITION	Brilliant Blue FCF consists essentially of disodium alpha-(4-(N-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-3-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene-2-sulfonate and its isomers and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. Brilliant Blue FCF is described as the sodium salt. The calcium and the potassium salt are also permitted.
CLASS	Triarylmethane
COLOUR INDEX NO.	42090
EEC NUMBER	E-133
EINECS	223-339-8
CHEMICAL NAMES	Disodium alpha -(4-(N-ethyl-3-sulfonatobenzylamino) phenyl)-alpha-(4-N-ethyl-3-sulfonatobenzylamino, cyclohexa-2,5-dienylidene) toluene-2-sulfonate
CHEMICAL FORMULA	<chem>C37H34N2Na2O9S3</chem>
MOLECULAR WEIGHT	792.84
ASSAY	Content not less than 85% total colouring matters, calculated as the sodium salt; E 1% 1cm 630 at ca 630 nm
DESCRIPTION	Reddish-blue powder
IDENTIFICATION	
a. Spectrometry b. Blue solution in water	Maximum in water at ca 630 nm
PURITY Water Insoluble matter	Not more than 0.2%

Subsidiary colouring matters	Not more than 6.0%
Organic compounds other than colouring matters	[Sum of 2-, 3- and 4-formyl benzene sulfonic acids] - Not more than 1.5% [3((ethyl)(4-sulfophenyl)amino) methyl benzene sulfonic acid] - Not more than 0.3%
Leuco base	Not more than 5.0%
Unsulfonated primary aromatic amines	Not more than 0.01% (Calculated as aniline)
Ether extractable matter	Not more than 0.2% at pH7
Arsenic	Not more than 3 mg/kg.
Lead	Not more than 10 mg/kg.
Mercury	Not more than 1 mg/kg.
Cadmium	Not more than 1 mg/kg.
Heavy metals (as Pb)	Not more than 40 mg/kg.

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